of the NorA model enabled the virtual screening of selected ligands from the ZINC database, and 14 hit molecules with adequate molecular properties and acceptable predicted toxicity were identified as potential candidates for EPI development (Bhaskar et al. 2016).

The limited availability of the 3D structures of efflux pumps in Gram-positive bacteria may be a reason for limited use of the structure-based approach to design EPI for NorA efflux pump. On the contrary, the availability of the resistance-nodulation-division (RND) efflux pump structures provided a good basis for studying the molecular mechanism of efflux pump inhibition in Gram-negative bacteria and identification of novel EPIs.

A reduced model of acriflavine resistance protein B (AcrB) without transmembrane domains (PDB entry: 4DX5) and in the absence of other accessory proteins, such as AcrA and AcrZ, was used to evaluate the interactions with the compounds MBX2319 (a novel pyranopyridine EPI), D13-9001, 1-[1-naphthylmethyl]-piperazine, and MC-207,110. Molecular docking of these molecules into AcrB binding site was used to generate starting points for molecular dynamics simulations of complexes to consistently compare binding modes of these molecules and ability of the efflux pump to accommodate different substrates. It was confirmed that all inhibitors act most likely via competitive binding into active sites for substrates and possibly preventing conformational changes necessary for efflux to happen (Vargiu et al. 2014). Similarly, a set of 2-substituted benzothiazoles achieve a reversal in the antibacterial activity of ciprofloxacin with up to 10-fold lower MIC values. These compounds were docked into AcrB binding site (PDB entry: 2DRD) indicating that the tested compounds establish possible binding interactions with the phenylalanine-rich region in the distal binding site (Yilmaz et al. 2015).

However, to avoid a possibility of inhibitors to become substrates of the efflux pump, efflux substrate-like features could also be considered as an exclusion criterion to enrich the inhibitor identification process. A library of phytochemicals that did not contain pharmacophoric features of efflux pump substrates was screened using high-throughput docking against AcrB (PDB entry: 1T9Y) and MexB (PDB entry: 2V50) proteins. The two of the best hits from the screening, lanatoside C and daidzein, were validated as potential EPI by checkerboard synergy assay and ethidium bromide accumulation assay, achieving a good correlation between *in silico* screening and positive efflux inhibitory activity *in vitro* (Aparna et al. 2014).

Other components of the RND efflux pump, such as AdeB from the MDR strains of *A. baumannii*, were also considered as possible targets for EPI identification. A homology model was build using the AcrB multidrug efflux pump template (PDB entry: 1oy6) and used as a target for high-throughput virtual screening of 159 868 compounds. The compounds were filtered initially based on their predicted molecular properties and toxicological profile followed by two-stage docking process (standard and extra precision). This resulted in 123